

Table 1. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm loxP)

| Plasmid combo | μ g DNA | Plaques/dish (293 cells) | (Totals) | Plaques/dish (293Cre4 cells) | (Totals) |
|--|-------------|--------------------------|----------|------------------------------|----------|
| pCA36:pBHG10 | 5:5 | 0, 0, 0, 0 | | 0, 1, 2, 0 | |
| | 5:10 | 0, 0, 0, 1 | | 1, 0, 0, 0 | |
| | 10:10 | 2, 0, 1, 1 | | 1, 2, 0, 0 | |
| | | | (5) | | (7) |
| pCA36:pBHGloxΔE1,3 | 5:5 | 0, 0, 0, 1 | | 0, 0, 0, 0 | |
| | 5:10 | 0, 0, 0, 1 | | 0, 0, 0, 0 | |
| | 10:10 | 0, 0, 2, 1 | | 0, 0, 0, 0 | |
| | | | (5) | | (0) |
| pCA36lox:pBHG10 | 5:5 | 1, 3, 1, 0 | | 0, 1, 0, 1 | |
| | 5:10 | 0, 1, 0, 0 | | 0, 0, 1, 2 | |
| | 10:10 | 0, 0, 0, 0 | | 0, 1, 1, 0 | |
| | | | (6) | | (7) |
| pCA36lox:pBHGloxΔE1,3 | 5:5 | 1, 0, 0, 1 | | 15, 14, 20, 20 | |
| | 5:10 | 0, 0, 0, 0 | | 11, 15, 12, 16 | |
| | 10:10 | 0, 0, 1, 1 | | 18, 9, 10, 8 | |
| | | | (4) | | (168) |

Table 2. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ Vectors (\pm loxP)

| Plasmid combo | μ g DNA | Plaques/dish (293 cells) (Totals) | Plaques/dish (293Cre4 cells) (Totals) |
|--|-------------|-----------------------------------|---------------------------------------|
| pCA36:pBHGlox Δ E1,3 | 5:5 | 1, 1, 2, 6, 2, 3 (15) | 1, 1, 2, 1, 2, 3 (10) |
| pCA36lox:pBHGlox Δ E1,3 | 5:5 | 1, 2, 2, 2, 2, 1 (10) | 41,44,41,41,44,31 (242) |
| pCA36lox Δ :pBHGlox Δ E1,3 | 5:5 | 0, 0, 0, 0, 0, 0 (0) | 41,36,55,34,24,40 (230) |
| FG140 | 1 | 72, 72 | 150, 115 |

Table 3 Efficiency of Ad vector rescue by cotransfection with pBHGloxΔE1,3 and various shuttle plasmids^a

| Cell line | Shuttle plasmid | Plaques/dish | Average/dish |
|-----------|-----------------|----------------|--------------|
| 293 | pCA36lox | 6, 2, 3, 3, 5 | 3.8 |
| | pCA36loxΔ | 1, 4, 0, 0, 0 | 1.0 |
| | pCA36loxΔCreR | 2, 2, 4, 3, 2 | 2.6 |
| | pCA36loxΔCreT | 9, 4, 4, 7, 3 | 5.4 |
| 293Cre4 | pCA36loxΔ | 23, 28, 22, 28 | 25.3 |

^a 5μg of all plasmids were used in cotransfections.

Table 4. Efficiency of Ad vector rescue by cotransfection of 293 cells with pBHGloxΔE1,3 and shuttle plasmids encoding Cre^a.

| Cell line | Shuttle plasmid | Plaques/dish | Average/dish |
|-----------|------------------------------|--------------------|--------------|
| 293 | pCA36lox | 2, 3, 1, 0, 1 | 1.4 |
| | pCA36loxΔ | 1, 0, 0, 0, 0 | 0.2 |
| | pCA36loxΔCreT ^b | 3, 1, 5, 2, 4 | 3.0 |
| | pCA35loxΔCreITR ^b | 21, 20, 42, 34, 40 | 31.4 |

^aAll cotransfections performed with 5 μg of the indicated shuttle plasmid and 5 μg of pBHGloxΔE1,3

^bPlasmids illustrated in figure 8c.

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Table 5. Efficiency of rescue of fibre and E4 genes into Ad by cotransfection with pFG173lox and pFG23lox^a

| Plasmids | μ g DNA | Number of plaques (average/dish) | |
|---|-------------|----------------------------------|---------------------|
| | | 293 cells | 293Cre4 cells |
| pFG173lox ^b : pFG23dX1lox ^c | 5:5 | 0, 0, 0, 0 (0) | 33, 27, 39, 26 (31) |
| | 2:2 | 0, 0, 0, 0 (0) | 9, 15, 10, 9 (11) |
| pFG173 : pFG23dX1 | 5:5 | 0, 0, 0, 0 (0) | 0, 0, 1 (0.3) |
| pFG140 | 1 | 95 | 93 |

^aCotransfections as diagrammed in figure 9

^bDiagrammed in figure 9b

^cDiagrammed in figure 10

Table 6. Recombinant virus rescue following cotransfection of 293 cells with shuttle plasmids with or without a Cre expression cassette

| Plasmid | Number of plaques/dish (average/dish) |
|---------------|---------------------------------------|
| pCA36 | 1, 0, 0, 0 (0.3) |
| pCA36lox | 1, 1, 1, 0 (0.8) |
| pCA36loxΔ | 0, 0, 0, 0 |
| pCA36loxΔCreT | 2, 1, 2, 2 (1.8) |
| pFG140 | 40, 31 (35.5) |

293 cells were cotransfected with 5 μg of pBHGloxΔE1,3 and 5 μg of the indicated shuttle plasmid or 1 μg of pFG140

Table 7. Efficiency of Ad vector rescue by cotransfection of 293 cells with pBHG10 and shuttle plasmids with a single ITR or an ITR junction

| Plasmid | μg of DNA/60 mm | # of Plaques | Average |
|-----------------|-----------------|------------------|---------|
| pCA35:pBHG10 | 2:2 | 2, 0, 0, 1, 0, 2 | 0.83 |
| | 5:5 | 2, 2, 2, 1 | 1.75 |
| pCA35ITR:pBHG10 | 2:2 | 19, 11, 14, 12 | 14 |
| | 5:5 | 23, 23, 14, 17 | 19.25 |
| pFG140 | 1 | 96, 106 | 101 |

Table 8. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

| Shuttle Plasmid ^a | Plaques/dish (293 cells) | (Average) | Plaques/dish (293Cre4 cells) | (Average) |
|---------------------------------------|-----------------------------|-----------|---------------------------------|-----------|
| pCA36lox | 0, 0, 0, 0 | (0) | 13, 15, 3, 13 | (11) |
| pCA35loxITR | 8, 13, 21, 19 | (15) | 111, 131, 100, 130 | (113) |
| pCA36loxΔ | 0, 0, 0, 0 | (0) | 10, 8, 9, 12 | (10) |
| pCA35loxΔITR | 0, 0, 0, 0 | (0) | 91, 127, 141, 118 | (119) |
| pFG140 ^b | 75 | | 83 | |

^a All cotransfections 5 μ g shuttle plasmid + 5 μ g pBHGlox Δ E1,3

^b 1 μ g/dish

Table 9. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

| Plasmids | μ g DNA /dish | Number of plaques /dish (average/dish) | |
|--|-------------------|--|----------------------|
| | | 293 cells | 293Cre4 cells |
| pCA36:pBHGlox Δ E1,3 | 5:5 | ND | 0,3 (1.5) |
| pCA36lox:pBHGlox Δ E1,3 | 2:2 | ND | 9,3 (6) |
| | 5:5 | 2,0,0,0 (0.5) | 30,31,30,30 (30.25) |
| pCA35lox Δ CreITR:pBHGlox Δ E1,3 | 2:2 | ND | 71,60,56,79 (66.5)* |
| | 5:5 | 36 | 100,96 (98) |
| pCA35lox Δ ITR:pBHGlox Δ E1,3 | 2:2 | ND | 55,64,75,63 (64.25)* |
| | 5:5 | 0 | 120,113 (116.5) |
| pCA35loxITR:pBHGlox Δ E1,3 | 2:2 | ND | 53,54,61,66 (58.5)* |
| | 5:5 | ND | 130,126 (128) |
| pFG140 (DC) | 1 | 92 | 178 |
| pFG140 (CE) | 1 | 94 | 118 |

* 5 plaques picked from each of these cotransfections and analyzed. All + for β -gal and all had predicted viral DNA structure

Table 10. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

| Plasmids* | ugDNA/dish | Number of plaques/dish (average/dish) | |
|--------------------------|------------|---------------------------------------|--------------------------|
| | | 293 cells (average) | 293 Cre4 cells (average) |
| pCA36 | 5 | 1,0,0,0 (0.3) | 1,0,0,0 (0.3) |
| pCA36lox | 5 | 1,1,1,0 (0.8) | 10,18,6,7 (10.3) |
| pCA36lox Δ | 5 | 0,0,0,0 | 6,4,3,0 (3.25) |
| pCA36lox Δ CreT | 5 | 2,1,2,2 (1.8) | 4,4,2, (3.3) |
| pCA35lox Δ CreITR | 5 | 14,23,25,23 (21.3) | 116,79,83,100 (94.5) |
| pCA35lox Δ ITR | 5 | 0,0,0,0 (0) | 65,62,64,51 (60.5) |
| pCA35loxITR | 5 | 4,3,4,0 (2.8) | 114,101,75,79 (92.25) |
| pFG140 (DC) | 1 | 40,31 (35.5) | 106,92 (99) |
| pFG140 (CE) | 1 | 21,19 (20) | 44,42 (43) |

*cotransfections with 5 μ g pBHGlox Δ E1,3 except for pFG140

Table 11. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

Number of plaques/dish (average/dish)

| Genomic plasmid ^a | Shuttle plasmid ^a | 293 cells | 293Cre4 cells |
|------------------------------|------------------------------|--------------------|-----------------------|
| pBHGlox Δ E1,3 | pCA36 | 2,3,1,2 (2) | 3,3,3,1 (2.5) |
| | pCA36lox Δ | 0,0,0,0 (0) | 9,23,20,19 (17.8) |
| | pCA35loxITR | 26,27,15,12 (20) | 91,101,95,86 (93) |
| | pCA35lox Δ CreITR | 56,42,50,74 (55.5) | 94,90,96,92 (93) |
| pBHGlox Δ E1,3Cre | pCA36 | 1,1,0,0 (0.5) | 2,3,2,0 (1.8) |
| | pCA36lox Δ | 6,5,4,3 (4.5) | 20,14,28,24 (21.5) |
| | pCA35loxITR | 77,67,78,76 (74.5) | 125,120,130,135 (128) |
| | pCA35lox Δ CreITR | 40,46,47,34 (41.8) | 83,90,88,89 (87.5) |
| pBHGlox Δ E1,3CreR | pCA36 | 0,0 (0) | ND ^b |
| | pCA36lox Δ | 2,0 (1) | ND |
| | pCA35loxITR | 39,29 (34) | ND |
| | pCA35lox Δ CreITR | 7,6 (6.6) | ND |
| pFG140 | | 61,52 (56.5) | 85,87 (86) |

^a Cotransfections with 5 μ g each plasmid/dish except 1 μ g/dish for pFG140

^b Not done

Table 12. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

| Plaques / dish (average / dish) | | | | |
|----------------------------------|--------------------------|--------------|--------------------------------|-----------------------------------|
| Genomic plasmid | Shuttle plasmid | ug DNA /dish | 293 cells | 293Cre4 cells |
| pBHGlox Δ E1,3 | pCA36 | 5:5 | 2,2,1,0 (1.25) | 2,1,1,1 (1.3) |
| | pCA36lox Δ | 5:5 | 2,0,0,0 (0.5) | 26,28,25,27 (26.5) |
| | pCA35loxITR | 2:2 5:5 | ND ^a 3,9,6,6 (6) | 75,90 (82.5) TNTC ^b |
| | pCA35lox Δ ITR | 2:2 5:5 | ND 1,2,1,0 (1) | 55,64 (59.5) TNTC |
| | pCA35lox Δ CreITR | 2:2 5:5 | ND 33,28,35,31 (31.8) | 61,64 (62.5) TNTC |
| pBHGlox Δ E1,3 Cre | pCA36 | 5:5 | 2,2,1,0 (1.25) | 4,1,1,0 (1.5) |
| | pCA36lox Δ | 5:5 | 6,4,4,6 (5) | 21,25,21,17 (21) |
| | pCA35loxITR | 2:2 5:5 | ND 57,49,45,54(51.3) | 90,96 (93) TNTC |
| | pCA35lox Δ ITR | 2:2 5:5 | ND 39,45,39,46 (42.3) | 75,87 (81) TNTC |
| | pCA35lox Δ CreITR | 2:2 5:5 | ND 54,64,41,40(49.8) | 117,103 (110) TNTC |
| pFG140 | | 1 | 114,96 (105) | 125,140 (132.5) |

^a Not done

^b Too numerous to count

Table 13. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (CRE expressed from plasmids, by 293 cells, or both)

| Genomic plasmid | Shuttle plasmid | μ gDNA/dish | Plaques/dish (average/dish) | |
|--------------------------|--------------------------|-----------------|--|---|
| | | | 293 cells | 293Cre4 cells |
| pBHGlox Δ E1,3 | pCA35loxITR | 5:5 2:2 | 3, 6, 9, 13 (8) 6, 4, 1, 3 (4) | TNTC ^a (≥ 109) 65, 55, 64, 69 (63) |
| | pCA35lox Δ ITR | 5:5 2:2 | 0, 0 (0) 1, 0 (0.5) | TNTC (≥ 117) 49, 57, 47, 54 (52) |
| | pCA35lox Δ CreITR | 5:5 2:2 | 18, 21, 43 (27) 18, 12, 21, 24 (19) | TNTC (≥ 111) 74, 61, 50, 49 (59) |
| pBHGlox Δ E1,3Cre | pCA35loxITR | 5:5 2:2 | 52, 66, 63, 57 (60) 48, 47, 32, 43 (43) | TNTC (≥ 116) 72, 85, 69, 75 (72) |
| | pCA35lox Δ ITR | 5:5 2:2 | 40, 36, 32, 63 (43) 48, 43, 52, 46 (47) | TNTC (≥ 122) 93, 104, 106, 100 (101) |
| | pCA35lox Δ CreITR | 5:5 2:2 | 54, 56, 51 (54) 33, 37, 35, 19 (31) | TNTC (≥ 68) 110, 94, 89, 83 (94) |
| pFG140 | | 1 | 114 | 150 |

^a Too numerous to count